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BACTERIAL VARIATION[1,2]

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As far as consistent with an integrated presentation, this article will exclude literature discussed in three earlier reviews (5, 52, 61) with whose viewpoint the writer concurs. In particular, the exposition of the problem of directed adaptive variation has been fully discussed. Spontaneous mutation and natural selection are adequate to account for most adaptive changes in bacterial populations. From a general biological standpoint, exceptions to this rule would be very instructive, but except for induced lysogenicity, claims of lamarckian responses in bacteria have not been sufficiently fortified by experiment.

The classification of bacterial variations as mutations can be made only tentatively except where genetic analysis permits. Unfortunately, this is now possible only in strain K-12 of *Escherichia coli* which shows genetic recombination. A number of other mechanisms of heritable variations, summarized in Table I, are familiar to geneticists, and others may yet be established. Nevertheless, most of our information on bacterial variation is most conveniently organized in terms of mutation.

TABLE I

CATEGORIES OF GENETIC VARIATION

A. Changes which may occur in single cell cultures
  1. Mutation—chemical change or destruction of a hereditary particle
  2. Attenuation—quantitative changes in extranuclear units
  3. Segregation—from a heterokaryon or a heterozygote
  4. Chromosomal rearrangement and polyploidy
B. Changes involving extrinsic biological units
  5. Fusion of cells leading to heterokaryon or heterozygote
  6. Infection and transformation

BACTERIAL MUTATION STUDIES

Phage resistance mutants.—Quantitative mutation studies are easiest when the mutants can be counted by inspection. Mutations

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for resistance to bacteriophages have many virtues, as the number of resistant mutants in a population is estimated by plate counts of the survivors of phage attack. Infection and lysis of the cell is an all-or-none affair, and under appropriate conditions, every sensitive cell is destroyed, and every resistant survives. Account must be taken, however, of genetic variations in the phage stocks used (6).

In their classical work, Luria & Delbrück (62) found enormous fluctuations in the numbers of mutants in replicate cultures and showed that this argued against the direct induction of the mutations by the phage. This very high variance, compared to the sampling errors revealed by replicate assays from the same culture, shows that some factor not under experimental control is responsible for the fluctuations, and therefore for the mutations. That is to say, the mutations are spontaneous. The variance can be accounted for by the occurrence of the mutations throughout the development of the cultures. Those few which occur early will generate large clones of resistant cells, while the later ones will be multiplied by a smaller factor. The theoretical distributions of the mutants as a function of the mutation rate have not been published (see 28), but an approximate solution was given. With this method, the rate of mutation to resistance to T1 in *E. coli* B was estimated at 2×10^{-8} per fission cycle.

An independent estimate of the mutation rate, calculated from the fraction of cultures containing no mutants, gave 3×10^{-9} per fission cycle. A detailed investigation of this discrepancy led to the conclusion that mutant cells (first method) are produced about six times as rapidly as mutant clones (second method), which must mean that the mutant clones are initiated two or three generations before any descendant becomes phenotypically resistant. In other words, there is a variable lag between the occurrence of the genetic change and its phenotypic expression (74). Phenotypic lag may account for the fact that nondividing cultures do not accumulate mutants (62), and that disproportionate numbers of mutants appear during the first few divisions of a new growth cycle.

These rates are probably a sum for several distinct mutations. Selection for survivors of T1 attack leads to a variety of resistant types differing in nutritional requirements, colony morphology, and cross-resistance. The predominant types are /1, 5, or V_1^r, resistant both to T1 and T5, and /1, or V_{1a}^r, resistant only to T1.

Recombination tests in K-12 have proven them to be distinct mutations (50). There is some variation in the proportions of these main types in different stocks, but this may be due to epistatic effects as well as to differential mutation rates (6).

In strain B of *E. coli*, the /1 mutation is associated with a requirement for tryptophane (2), not yet observed in K-12. In a similar system, Wollman (103) has shown that tryptophane-independence can not be restored by reversion, but only by two mutational steps which leave the culture still resistant.

A third described type of resistant mutant is $V_{1b}{}^r$, an unstable mucoid form frequently found in K-12. Certain complex resistance patterns have also been described as occurring rarely (6, 23, 60). Since they can be pictured as the superposition of more frequent simpler patterns, they may not be simple gene changes, but "mass rearrangement of the genetic material of the bacterial cell, possibly comparable to that responsible for chromosomal rearrangements in higher organisms." Unfortunately, K-12 in which such a hypothesis could be tested has not yet produced such complex types.

The different resistance patterns described above probably depend on mutations at different loci. However, a type has been described, $V_{1c}{}^r$, which may be allelic to $V_1{}^r$ (54). $V_{1c}{}^r$ is partially resistant to T5, T1h, and T_1, increasing in that order. With $V_1{}^r$, no recombinations which would be completely sensitive were observed in 199 tests, but a diploid heterozygote which was discovered in this experiment proved to be sensitive. Thus $V_1{}^r$ and $V_{1c}{}^r$ are either allelic or closely linked. In any event they have a complementary action since the combination of the two mutants showed the wild, sensitive, phenotype.

Mutation to phage resistance, which occurs spontaneously, may be accelerated by the use of various mutagenic agents, preeminently radiations (24). The most striking feature of this work is an apparent delay in the expression of the mutations which are induced either by x-rays or by ultraviolet light. Phenotypic lag, as discussed for spontaneous mutations, accounts for part of this delay. In fact it is difficult to explain the induced zero-point mutations which are detectable immediately after irradiation. Radiations may have a direct accelerating effect on phenotypic lag, perhaps by destroying the residual receptors which are responsible for sensitivity. On the other hand, the induced zero-point mutations may be partly artefacts. Such mutations are produced in

appreciable numbers only by doses which kill most of the bacteria. The killed bacteria, however, are still able to absorb, but not to regenerate phage. When present in excess, they may protect sensitive bacteria from infection long enough to allow phenotypic lag to run its course. According to Beale (6), there should be a severalfold excess of phage over bacteria to ensure immediate lysis of sensitive cells, and if the sterilized bacteria are taken into account, this condition was not always fulfilled in the published experiments.

However, the delay in induced mutations lasts at least three times as long as the two or three generations of phenotypic lag. The discrepancy may well be due to segregation since cells of *E. coli* are typically multinucleate (80). Phage sensitivity is dominant to resistance in heterozygotes, so that one would anticipate that resistance mutations can come to expression only when there are no sensitive nuclei in the same cell, after enough cell divisions to segregate the mutant nuclei. In *Neurospora tetrasperma*, radiations have a haploidizing effect on heterokaryotic ascospores, and possibly in bacteria (see 20, 52), although the first order kinetics of sterilization of *E. coli* seems to preclude a dominant role for such an effect (102).

Phage resistance mutations have been used to test a variety of chemical compounds for mutagenic activity. Substituted *bis*-(2-chloroethyl)-amines, nitrogen mustards, are outstanding in this respect, and closely simulate the action of radiations both in higher organisms (3) and in microorganisms (14, 17, 93). Of the other compounds tested, sodium desoxycholate has been scrutinized most closely (101). Eight hours exposure of *E. coli* B to a 5 per cent solution at pH 7.7 had a mutafacient and lethal effect equivalent to 100,000 r of x-radiation. The induced mutations were measured only as zero-point mutations, but in view of the long duration of the treatments, the conditions are hardly comparable to those in which radiation was used. Very thorough measures were taken to control possible errors based upon selective survival of mutants as against wild-type cells during the treatment, especially by reconstruction experiments using artificial mixtures of sensitive and resistant cells. However, it would be very difficult to devise controls for a simulation of mutagenic activity by the disclosure of mutations which had occurred previously, but which were camouflaged either by phenotypic lag or by genetic dominance.

From the *E. coli* evidence alone, it could not be demonstrated quite conclusively that sodium desoxycholate is mutagenic, but the report that this compound has a similar effect in *Drosophila* may be taken as a confirmation (101). The mutagenicity of desoxycholate in *E. coli* has been confirmed by Latarjet (48), who reports that cholate is likewise effective.

Among other compounds tested, pyronin Y, Styryl 430, 1,2,5,6 dibenzanthracene endo-succinate, alkyl urethans, caffeine and colchicine, and sodium chloride in high concentrations, are reported to be mutagenic while methyl green, methylcholanthrene, and methylcholanthrene photoöxide are inactive (13, 48, 101). The penetration of the latter two materials into the cell was shown by fluorescence.

Biochemical mutations in bacteria.—Mutations affecting biochemical processes are interesting chiefly for physiological studies, because their application to quantitative mutation studies is limited by technical difficulties. However, a number of artifices have been described which facilitate the isolation of biochemical mutants (19, 29, 55). The most efficient of these utilizes the specific bactericidal action of penicillin on cells capable of growth to eliminate wild type cells inoculated in synthetic medium, while saving the mutants (20, 56).

Some of the biochemical problems which have been attacked recently, using mutants, include sensitivity to penicillin in *Salmonella* (78), carbon and nitrogen metabolism in *Azotobacter* (43, 59) [see also (100)], bioluminescence in *Achromobacter fischeri* (67, 34), the metabolism of proline, phenylalanine, tryosine, and their peptides (32, 87, 88), and of maltose in *E. coli* (27).

Hinshelwood & Peacocke have objected to describing these biochemical variants as mutants (77). After ultraviolet treatment, they isolated 48 colonies of *Aerobacter aerogenes* and studied their growth behavior in artificial media. In some of the tests, a prolongation of lag was observed, but this disappeared after subculture on a complete medium. From the behavior of these cultures, they conclude that biochemical mutants represent merely temporary alterations in the enzyme balance of the cells, reparable by a direct adaptation or training process. It will be agreed that whatever Hinshelwood's cultures were, they probably were not mutants. It is not surprising that no mutants were isolated in these experiments in view of (*a*) the limited number of colonies that were tested and

(b) the absence of a period of proliferation after the treatment to take account of the delayed effect.

The present writer has occasionally isolated cultures of E. coli like Hinshelwood's, which might be called transitory mutants (see 55, 56), and they have also been seen in Neurospora. Hinshelwood's interpretations may well account for some of these, but reverse mutation must also be considered. However, these ill-defined isolates should not be confused with the clear-cut mutants with reproducible, specific, nutritional requirements that are used in genetic and biochemical research.

In contrast to the difficulties of estimating rates of mutation to biochemical deficiency, reverse mutations are rather easily determined, by inoculating washed suspensions into synthetic media, and counting the colonies which develop.

Many biochemical mutants in E. coli exhibit spontaneous reversion rates ranging from about 10^{-9} to 10^{-6} per fission cycle. However, reverse-mutations of nutritional requirements in bacteria have not yet been studied genetically to prove that the phenotypic reversion is based on reverse rather than suppressor mutation.

Ryan & Schneider have made detailed measurements of reversion rates in several coli mutants, especially one requiring histidine (82, 83). The adaption of this histidineless mutant to growth on minimal medium is complicated by a selective interaction which depends on the histidine concentration. At minimal levels of histidine the growth of histidineless cells is impaired, while wild-type histidine-independent cells grow freely. At optimal levels, both types of cells proliferate freely, and mixed cultures do not change their composition during growth. At intermediate levels, however, growth of the wild type cells is suppressed, and in mixed cultures with histidineless, the proportion of histidine-independent cells may remain nearly constant. At the time of writing, the series of publications recounting this interesting phenomenon had not been completed. However, it appears likely that the selective phenomena brought to light by the reconstruction experiments account fully for the course of events when the histidineless mutant is inoculated into synthetic media with various levels of histidine. With no histidine, some of the cultures may eventually adapt, and reach a final level of growth equal to that of the wild type in the same medium. The adapted cultures contain a preponderance of cells which are thereafter independent of histidine, and are presumably

the result of selection of a small proportion of spontaneous reversions from histidineless. When intermediate levels of histidine are used, the adaptation is suppressed, and in all of the cultures, the ultimate density is proportional to the amount of histidine added. It is to be supposed that the limiting level of histidine influences the course of selection rather than the rate of the mutation which underlies the adaptation. With optimal levels of histidine, of course, there is no selective pressure to favor the reverse-mutants, and these accumulate only to a negligible extent under mutation pressure. Although the biochemical basis of these selective interactions has not yet been revealed, a parallel example in *Neurospora*, with a more thorough genetic analysis, has been discussed (81).

Guthrie has presented preliminary data on the reversion of a purine-less coli which he interpreted as a direct effect of the medium on the reverse mutation rate (36). However, he now inclines to an interpretation based on modification of selection dynamics, very similar to Ryan's conclusions (37). This type of selective interaction probably accounts also for other examples of an environmental effect on genetic adaptation, e.g., the reversion of the requirement for tryptophane in lactobacilli (104).

The training of typhoid bacteria to dispense with tryptophane is best understood as an example of reverse-mutation (30, 33), although, fortunately, the complications noted with histidineless coli have not been observed.

The term adaptation has always been used rather loosely. To the general biologist, it means only a change in an organism or species which seems to result in a better fit to its local environment. It should not connote the mechanisms by which it is accomplished. In microorganisms, adaptation is often genetic, i.e., is the result of an inherited variation which occurs spontaneously, but which becomes established under the pressure of natural selection just because it results in greater fitness. In Protozoa it seems to be established that an inherited adaptation may sometimes be the result of a direct reaction with an environmental factor (89). However, there is no convincing evidence, as yet, for such an example in the bacteria, except for induced lysogenicity. There are, of course, many examples of direct adaptation, viz., enzymatic adaptation to substrates (where it occurs in nongrowing cultures and natural selection can be excluded), or to changes in salt concentration. However, such adaptations are generally not inherited

and quickly disappear upon the removal of the inciting agent. Direct heritable adaptations may be a reflection of cytoplasmic heredity, a subject of very great interest in genetics today, which is all the more reason why possible examples should be scrutinized most thoroughly, especially to disqualify natural selection.

The biochemical changes which are subsumed under nutritional mutations are, unfortunately, very difficult to analyze enzymatically, although an encouraging start has been made in *Neurospora* with systems involved in the synthesis of tryptophane and of pantothenic acid (71, 97). For studies on gene action in bacteria, effects on the enzymes involved in carbon metabolism should be more easily investigated by the biochemist.

Many descriptions of fermentative variations appear in the literature, dating from Massini's classical description of *E. coli mutabile*. They are readily produced under the influence of mutagens, and easily detected with the help of indicator media such as Levine's Eosin-Methylene Blue Agar (57), or with triphenyl tetrazolium (53). When 10^8 cells of wild type *E. coli* are spread on an indicator agar plate, and exposed to ultraviolet light long enough to reduce the survivors to about 200 to 400 colonies, a yield, e.g., of lactose negative mutants of 1 to 1,000 to 1 to 5,000 is found by inspection. Mutants discovered in this way are, as often as not, in the form of sectors rather than intact mutant colonies. The sectored colonies consist of mutant and nonmutant components, and it seems reasonable to ascribe them to the segregation from a bi- or multinucleate cell, in only one nucleus of which a mutation had occurred. Barring only the contingency that the sectored colonies arose from two cells close together which happened to survive the heavy dose of radiation, they afford direct evidence of such a segregation process, which is, after all, only to be expected from the cytological evidence, if the desoxyribonucleic acid containing bodies in the cell are accepted as nuclei.

Kristensen's studies of fermentative variation in *Salmonella* (47) have shown (a) that mutations leading to the ability to ferment a given sugar are spontaneous, and merely selected for by the presence of the sugar, and (b) that mutations concerning different sugars are usually independent of each other. However, in *S. typhosa* Type II, one form is inhibited by xylose and ferments dulcitol; the alternative is not inhibited by xylose and fails to ferment dulcitol. He has been able to demonstrate mutation from

one to the other by using xylose and dulcitol media respectively. Unfortunately, this system is not well adapted to accurate measurements of mutation rates in the two directions.

Monod (72, 73) has isolated lactase from wild type *E. coli* and has demonstrated that it is a simple hydrolytic enzyme, strictly adaptive, and absent in mutants which are unable to ferment lactose. He has also compared bacteria whose utilization of other sugars had been intensified by selective culture and found no differences in their capacity to oxidize lactose. He concluded that the formation of lactase was under unitary genic control, i.e., that the enzyme was produced under the influence of one and only one gene. This useful working hypothesis has had wide circulation as the one-to-one theory (8). However, the present writer's experiments have not been in such good accord with it (51). Among several hundred lactose negative mutants at least seven distinct classes have been identified which differ by mutations of different genes, as determined by recombination tests. Two of these classes have alterations in enzymes other than lactase, both being unable to ferment maltose; one glucose negative (27), the other gluconate negative. Since the specificity of adaptation shows that lactase must be distinct from these enzymes, these mutations are pleiotropic, i.e., they influence several enzymes. For these adaptive enzymes, it seems likely that there is not a one-to-one relationship between any gene and the enzyme finally produced, but that the gene impinges on the complex adaptation mechanism. Some of the mutants will produce lactase under conditions of altered temperature or substrate, which is hardly consistent with the hypothesis that the alteration of the gene means the absence of the specificity of the enzyme. For the possibly more direct synthesis of constitutive and biosynthetic enzymes, a simpler relationship between gene and enzyme may hold.

Fermentative mutants, whose stability can be assayed by inspection of colonies, are favorable material for the study of the genetic control of genetic stability (49). Some differences in the reverse-mutability of various lactose negative mutants in *E. coli* are due to allelomorphs of different stability. Both spontaneously, and under the influence of ultraviolet light, derived mutants with lower reversion rates were found, but increased mutability was not. The apparent stability of certain derived stable lines depends on the accretion of a second mutation interfering with the utilization

of lactose. In such a double mutant, the expression of a reversion of either mutant gene is prevented by the other.

Mutations affecting colony color, although having an obscure biochemical basis, are convenient for certain types of mutation and population studies. With such variants in *Phytomonas stewartii*, Lincoln (58) has evaluated the role of mutation and selection in the effects of temperature on the variation of bacterial populations. Temperature has only a relatively small influence on mutation rate, two- or three-fold increases for a rise of 10°C., as in higher organisms, compared to its profound effects on the selective advantages of various types in mixed cultures. Since, according to the most popular theory, spontaneous mutations are thermal accidents, quantitative studies on the effect of temperature changes and temperature shocks on mutation rates assume considerable importance.

Drug resistance mutations.—Mutations conferring resistance to antibacterial agents are of special interest to medical bacteriologists. That such mutations may be induced by contact with the drug is still widely believed, but there is no convincing evidence to substantiate it. Statistical analyses of penicillin (60), sulfonamide (75), and streptomycin (22) resistance in staphylococci and in *E. coli*, along the lines discussed for phage resistance in *E. coli*, support the concept of spontaneous mutation and selection.

Drug resistance is often relative or quantitative rather than sharply qualitative. Firstly, resistance refers to a definite concentration of the drug, and there is often a very sharp cut-off in the proportion of surviving cells with relatively small increases in drug concentration. Secondly, resistance may be variably expressed in a population, to be described as a distribution rather than a single parameter. The distributions of adjacent steps of resistance may overlap. Therefore, experiments on these mutations must be closely controlled to insure that effects on the expression of resistance are nullified.

The metabolism of resistant mutants may be altered, but enzymatic changes are not necessarily the genetic cause of the resistance. But a number of workers have interpreted resistance as the direct injury of cellular enzymes by the drug (18, 84). Since these metabolic changes persist on cultivation in drug-free medium, it would have to be argued (40) that the susceptible enzymes are

autocatalytic, i.e., that the alteration is transmitted in a heritable
fashion. This conclusion needs more convincing evidence than is
now at hand to substantiate it over the gene theory of inheritance.
Genetic resistance is, of course, probably mediated by effects on
enzymes, but this conclusion should not be confused with the hy-
pothesis of direct injury (see 61). Because the enzymatic mech-
anisms of antibacterial action are still largely inaccessible, there
has been relatively little work to show different responses of en-
zymes of resistant mutants to the antibacterial agent. Streptomy-
cin has been reported to inhibit benzoic acid oxidation in sensitive
mycobacteria, but not in resistant mutants. Further work (31),
however, showed that this effect was not on the oxidative enzymes
per se, but on their adaptive formation, concerning which as little
is known as about growth as a whole. Sevag & Gots have, however,
examined dehydrogenase activity in pneumococci and found evi-
dence for the occurrence of altered enzyme proteins in mutants
resistant to a variety of inhibitors (85).

A number of bacteria develop a requirement for streptomycin
(70, 76) concomitantly with resistance. Animals injected with
streptomycin-dependent meningococci will survive unless they are
treated with streptomycin. The biochemical basis of this require-
ment is as obscure as the mode of action of streptomycin, but is
perhaps clarified by experiments on sulfonamide requiring *Neuro-
spora* (108). In this fungus, sulfonamide resistance is sometimes
associated with dependence on sulfonamide. If, however, an addi-
tional mutation is introduced which prevents the synthesis of
p-aminobenzoic acid (PAB), sulfonamide is not required unless
PAB is added in too large amounts. Apparently, resistance to sul-
fonamides was effected by a very efficient utilization of PAB, and
a sensitivity to excess of it. Normal synthesis of PAB exceeds the
sensitivity threshold, so that growth is inhibited unless the PAB
antagonist, the sulfonamide, is also added. In streptomycin de-
pendence we may likewise imagine that the resistance mutation
is accompanied by an expansion of the sensitive enzyme systems
so that they are in balance in the presence of the inhibitor; when
uninhibited, their exaggerated activity may be supposed to inter-
fere with normal growth.

Genetic resistance may make growth insensitive to an inhibitor,
but leave other processes liable to interference. For example,

mucoid *Brucella abortus* yields mutants whose growth is not affected by streptomycin, but which have a rough rather than a mucoid appearance in its presence (41).

Resistance mutations in staphylococci have been used by Stone, Wyss *et al.* (90, 91, 106, 107) to test for indirect mutagenic effects of radiation. They find that nutrient broth, heavily irradiated with ultraviolet light, induces penicillin resistant mutations. Possible inaccuracies due to selection of pre-existing mutants have been controlled experimentally. Irradiated broth also increased the rate of mutation both to mannitol negative from the normal mannitol positive, and the reversion back to positive. The effects of radiation on broth can be duplicated with hydrogen peroxide, and both treatments can be negated with catalase. However, peroxide applied directly to washed cells has no mutagenic action, and it was therefore concluded that peroxide reacts with some component of the broth to form the mutagenic compound. These experiments pose some interesting problems for the direct effects of radiation. However, they do not, as yet, provide unequivocal support for the hypothesis that "modified substrate molecules may be assimilated by the organism and built into inexact replications of the genetic mechanism." This hypothesis is to be compared to the conception that mutagens act in some way to increase the non-specific activation energy for chemical change of the gene. The latter notion, long current for radiations, should be extended to nitrogen mustard, because this compound induces reverse-mutations as well as mutations (35). But irradiated broth also increases the rate of reverse-mutation, if, as these authors suggest, the mutation from mannitol negative to positive is to be so regarded. It is difficult to see how a second inexactitude in the replication of a gene, in the specific way envisaged above, could reverse the effect of a first. At any rate, it should be possible to test the hypothesis of analogue assimilation into the gene by thorough comparisons of mutation and reverse mutation rates under the influence of these mutagens, preferably in several diverse systems.

Many studies of direct adaptive resistance allow a prima facie case for natural selection which is at least as good as for the direct response. However, Strandskov (92) has reported some observations on resistance of *E. coli* to 2-chloro-PAB (CPAB), which need a more thorough analysis. The most critical experiment involved the plating of a small number of sensitive cells on CPAB-agar.

After four days, 10 to 25 per cent of the cells developed into colonies which consisted of resistant cells. Since there was clearly no such high proportion of resistants in the original population, it could be inferred that the resistance was induced by the CPAB. It was recorded that resistance was retained through one year of culturing on nutrient agar, i.e,. that it was transmissible. However, it has since been observed (unpublished work of the writer) that the resistant colonies do not develop directly from single cells, but that microcolonies of some thousands of cells form initially in the inhibitor medium. These microcolonies, invisible to the naked eye, open the door to natural selection by providing populations in which spontaneous mutations can occur. It should be emphasized that this observation does not prove natural selection, nor does it disprove direct adaptation, but it does leave the question open for further study.

Resistance to physical agents.—Mutations augmenting bacterial resistance to physical agents have also been found. In *E. coli* B, a mutant, B/r, with increased resistance to x-rays and ultraviolet light has been found among the survivors of heavy doses of such radiation (102). Since the mechanism of radiation killing is still under discussion, there is no clear interpretation of the resistance noted in B/r. According to Demerec & Latarjet (24), the mutational response to irradiation is the same in B and B/r for a given physical dose. The changed response to x-rays is in the form of a flattened slope of the log survivor/dose curves, while with ultraviolet the linear relationship, seen on B, changes to a sigmoid response on B/r, indicating that several cumulative hits are required to kill a B/r cell. However, the lethal response of B, both to x-rays and ultraviolet, breaks sharply toward resistance at about 1 per cent survival. This break cannot be accounted for in terms of resistant mutants (17), but is, on the other hand, inconsistent with the single-hit theory of radiation sterilization.

In a very recent report, Kelner (45) has indicated that the bactericidal effects of ultraviolet light can be substantially reversed with visible light. Photoreactivation occurs in actinomycete spores, bacteria, and bacteriophage, so that this observation may lead to a significant extension of our understanding of radiobiological effects.

Breaks in the lethal responses of bacteria to sterilization with heat have been frequently noted, but only rarely has the question

of the heritable resistance of the survivors been considered. In *E. coli*, the resistant tail of the distribution of responses to heat does not give rise to a more heat resistant culture when grown out, and the prolonged survival of these cells must be presumed to originate in their physiological condition at the time of treatment rather than in genetic differences (42). However, spores of *B. subtilis* which survived thermal inactivation produced progeny with augmented resistance to heat (21). These observations recall the tolerance to heat which Kluyver & Baars succeeded in developing in sulfate reducers by gradual acclimatization (46). Although they supposed it to be a physiological adaptation, the evidence does not exclude mutation and selection.

The response of *E. coli* to osmotic pressure changes may involve both physiological adaptation and genetic changes (26). By increasing the salt concentration of the medium gradually, most of the cells in a resting suspension can be acclimatized to high salt concentrations; on the other hand, the survivors of sudden changes in concentration give tolerant progeny.

Antigenic variation.—Space does not permit of adequate consideration of the extensive research on *Salmonella* antigenic variation which fortunately, however, has been reviewed recently by Harrison (39). Since then, Bruner & Edwards (10) have demonstrated, with the help of antiserums, changes from the nonspecific 1, 2, 3 . . . phases of a number of types to a 1, 2 . . . phase characteristic of other named types. Therefore, they (11) and Kauffmann (44) have recommended that the 3 antigen be dropped from the diagnostic schema, and that the types which were formerly distinguished by it be coalesced with the 1, 2 . . . named types. In another report (12), they demonstrated induced changes in the somatic antigens. In the presence of X, XXVI antiserum, the homologous component in *S. anatum* and in *S. meleagridis* was replaced by the XV antigen, yielding forms indistinguishable from *S. newington* and *S. cambridge* respectively.

The role of antiserum in these experiments has not been settled definitely, but no compelling reasons have been offered to regard it as other than selective for spontaneously occurring variations.

Serological work on these organisms has proceeded so much further than our understanding of the genetic mechanism involved that it would be fruitless to offer any ready-made explanations. The dimorphic phase variation of flagellar antigens is especially

perplexing, but it may be useful to look for an interpretation in the light of recent studies of the cytoplasmic determination of antigens in *Paramecium* (89).

Braun (9) has reviewed his studies of dissociation in *Brucella*. This work has provided strong support for the role of spontaneous mutation and selection in determining the nature and extent of population changes. Intrinsic differences in the dissociation index were shown to be explicable in terms of growth responses of the original smooth strains, and their various mutants, as could the influence of such environmental factors as temperature, pH, renewal of the medium, and a serum factor. However, it is not clear whether the failure of certain strains to produce the so-called S^r type is due to intrinsic differences in the range of genetic variability, or to selective factors which operate after variations have occurred.

Not all recent students agree that apparent effects of environment on rates of dissociation are due to selective interactions acting after the spontaneous appearance of the mutant. In *Brucella bronchiseptica*, Dickinson noted (25) that variation of a culture from N, normal, to V, a relatively avirulent variant, was suppressed either by a deficiency for chlorides, or by the addition of maleic acid as a carbon source in the presence of adequate chloride. In an attempt to reconstruct the populational relationships, the effect of maleate on the composition of mixed N-V cultures was examined. "In Koser's medium even the weakest mixture became pure V within three or four subcultures," showing that there is a strong selective advantage here. "In chloride free medium mixtures containing up to one loopful of V did not reveal V colonies after twenty subcultures but mixtures containing two loopfuls or more of V became pure V. . . . " This statement shows that chloride strongly influences the selective advantages of V. But the conclusion that maleate affects the mutational process per se, and not selection, cannot be inferred from "In maleate medium, with and without chloride, the weakest mixture tested contained one loopful of V and this rapidly became pure V," since it had been established that "one loopful of V" is a critical density of cells, above which V will predominate in chloride-free medium, and below which it will not. Before conclusions as to the behavior of newly produced variants may be drawn from reconstruction experiments, such experiments must be performed under conditions approxi-

mating as closely as possible those of the variation experiment.
This work is quoted at such length because it is a remarkably good
example of a difference in the behavior of a population at different
initial concentrations. Although it is not established that these
salts influence variation per se, it is no less interesting that such
materials can so profoundly influence the behavior of a population.

A report (68) whose evaluation is less certain now concerns the
influence of acetate on mucoid to smooth variation in a group C
hemolytic streptococcus. This organism is unstable in medium
lacking acetate, and cultures rapidly become smooth after three
to six transfers, although the mucoid form is perpetuated in the
presence of acetate. The smooth variants are stable, not reverting to
mucoid during 14 transfers in acetate medium, so that we have a her-
itable variation, not an environmental effect. In order to dispose of
selection "two single S phase cells were mixed in acetate-containing
broth containing 5,000,000 M phase cells. After eighteen hours incu-
bation at 37°C. equal numbers of M and S forms were present." The
authors therefore conclude that whether acetate is present or not,
the S form has such a powerful selective advantage that, if formed,
it should predominate. This is certainly the kind of evidence which
should be adduced in support of their hypothesis, but it is regret-
table that a more complete study of the population dynamics
has not yet been made. It may be pointed out that the S phase
consists of long chains of cocci, so that many more than two cells
were undoubtedly introduced in this limited reconstruction ex-
periment. It is interesting to notice that S has such a selective ad-
vantage in view of the remark that "the growth rates of (S and M)
in both . . . media were identical. . . . " Such interactions not
explainable in terms of the growth rates of the isolated cultures
are quite common. However, these combinations can be expected
to be the most unstable, especially with respect to the proportion
of cells of the variant which is initially needed for it to predominate.
If further work confirms this response as induced directly by the
absence of acetate, it would probably be best explained as deple-
tion of a cytoplasmic factor, like the attenuation of kappa in
Paramecium (79). Kinetic studies on the rate of transformation of
individual cells and their progeny in acetate free medium would
then give considerable insight into the mechanism. However, it
still seems an open question to this reviewer whether these results
are best accounted for by direct induction or by spontaneous muta-
tion and selection.

It would seem that there are no well substantiated examples of direct adaptation in bacteria. However, the gradual attenuation of virulence in *Phytomonas tumefaciens* cultured on glycine, resulting ultimately in an irreversible loss of pathogenicity, may be a promising example of the quantitative modification of a cytoplasmic genetic factor (95).

"INFECTIVE TRANSMISSION": TYPE TRANSFORMATION AND INDUCED LYSOGENICITY

The story of type transformations in pneumococci must be familiar by now to all readers of these reviews. Since last surveyed (66) there has been only one report dealing with this system. MacLeod & Krauss (64) have found an intermediate Type II, which is visibly acapsulate, but which produces serologically detectable specific polysaccharide. Competent rough strains are transformed to this intermediate smooth under the influence of polymeric desoxyribonucleic acid extracted from it. The intermediates were susceptible to further transformation to the typical smooth Type II, but unlike the roughs from which they were derived, could not be transformed into other smooth strains. In addition, the intermediate to smooth II transformation was not brought about by transforming principles isolated from other pneumococcal types. Evidently we are not dealing with a simple "intensifier" factor. It is not yet known whether transforming factors operate in pneumococci for characters other than the capsular polysaccharide. It may be recalled that the host specificity for rabbits of a pneumococcus strain was invariant under capsular transformation, suggesting a "somatic" basis for this character, while pathogenicity for mice in other types was correlated with transformation to and from type XIV polysaccharide, and is apparently connected directly with it (65, 86). No further evidence has been brought to bear on the question, raised primarily by Mirsky, as to the sufficiency of the desoxyribonucleic acid component of the transforming principles, and whether protein contaminant is responsible for its specificity. In this connection, a detailed publication of the data mentioned by Avery *et al.* (4) on the electrophoretic and ultracentrifugal homogeneity of the active preparations, and the size and shape determinations would be desirable.

There have been several further reports of transformations in other bacteria, without enough details to permit of an analysis. Wyss (105) and Vourcka (96) have reported that staphylococci

and *E. coli* grown in the presence of extracts of drug resistant bacteria acquired a heritable resistance to the drugs concerned. However, there might have been a physiological potentiation of resistance followed by spontaneous mutation and selection in the populations thus permitted to develop. In analogy to the pneumococcus transformation, a conversion of a nonmotile *Bacillus mesentericus* to a motile *B. anthracis* is reported (69).

Our present information on transformations does not allow any reliable judgments on their bearing on the genetic processes observed more usually in higher forms. The more credible reports uniformly picture the acquisition of a genetic function, and from purely mechanical considerations it would seem most likely that the transforming agents are incorporated into a cytoplasmic system like that of kappa to perform such functions. There would also seem to be a parallelism with the phenomenon of induced lysogenicity, which has been pointed out before, but which deserves greater emphasis, especially in view of the apparent justification of Altenburg's "viroid" theory of kappa (1).

Although most encounters between a phage particle and a "sensitive" bacterium result in the destruction of the cell, certain combinations may result in the establishment of a symbiotic relationship, whereby the phage multiplies without apparent injury to the bacterium, and on the other hand, the bacterium may acquire resistance to other particles of the same or other phages, presumably by the "interference effect" (15). Very little is known of the critical conditions which determine whether lysogenicity or lysis will be the issue, except that in *Bacillus*, lysogenicity may be related to the adsorption of phage just prior to sporulation of the bacteria. Once established, it appears to be very difficult to disinfect the phage, as Burnet states, "The permanence of the lysogenic character makes it necessary to assume the presence of bacteriophage or its anlage in every cell of the culture, i.e., it is part of the hereditary constitution of the strain" (16). Now, with respect to resistance to other phages, a lysogenic bacterium has a cytoplasmic genetic determination of this character, and one, moreover, which is capable of transformation by the use of extracts, i.e., preparations of the lysogenic phage. This is shown especially well in studies on phage typing of staphylococci, in which the primary determination of resistance patterns seems to depend on lysogenicity, genetic differences playing only a secondary role (99).

Many organisms are lysogenic as first revealed when they are tested on appropriate sensitive indicator strains (16). Since a limited number of indicator strains are used, it is quite possible that most bacteria carry these symbionts. The activity of these agents may well account for the transformations of virulence observed in *S. typhi-murium* (63) and possibly, if lysogenicity affects antigenic qualities, the transformations observed in *E. coli* (7) and in *Shigella* (98).

If the lytic activity of the symbionts were not apparent owing to the lack of suitable indicator strains, the incidental effects would have to be regarded as determined by a cytoplasmic factor. Furthermore, since the symbiotic phage is subject to mutation, and the bacterium-phage system to natural selection, one can speculate that functions ordinarily relegated to the nuclear mechanisms might be taken over by the symbionts, along the lines suggested by Altenburg and Darlington. But for this type of evolution in bacteria, proof is still wanting.

GENETIC RECOMBINATION

The question of a sexual phase in bacteria has been moot for many years, but genetic evidence to support it is now available for *Escherichia coli* K-12. Suggested by the occurrence of prototrophic recombinants in mixtures of complementary biochemical mutants (94) the hypothesis of sexuality was strengthened by a study (50) of the segregation of other characters, including sugar fermentations and phage resistance, in a way that indicated linear linkages. Some of these findings have been retested and confirmed (38). Finally, stocks have been described (54) in which a diploid heterozygote can be isolated, and allowed to segregate, yielding various recombination classes, as well as the character combinations of parents. The discovery of these heterozygotes would seem to make very remote any interpretation of recombination based upon transforming factors like those described in pneumococci.

It cannot be said how important recombination is in the genetic variation of other bacteria, for studies are still in progress to determine its occurrence elsewhere than in K-12. However, recombination can only reshuffle mutations which have already occurred, and is not a primary source of variation. Therefore, perhaps the most important aspect of genetic recombination to students of variation is as a tool for their analysis of bacterial variation.

Cytological evidence for processes of nuclear fusion is still controversial [see (80)]. It has not yet been possible to correlate cytological with genetic studies on any one organism, but with the material now available, we may look forward to the establishment of a bacterial cytogenetics.

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